Single Phage Suspensions and Phage Cocktail in the Inactivation of *E. coli* and *S.* Typhimurium: A Preliminary Study

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Introduction

- Enterobacteriaceae *Escherichia coli* and *Salmonella enterica* serovar Typhimurium strains are responsible for numerous infections, frequently presenting resistance to antibiotics.
- As treatment for Enterobacteriaceae infections is empiric, using the same antibiotics to treat *E. coli* and *Salmonella* infections, the same concept can be applied with phages.
- Combining different phages into cocktails circumvents the development of phage-resistant mutants and allows for the treatment of multiple pathogens, broadening the phage's action spectrum. [1]

Goal

Evaluate the efficiency of a two-phage cocktail (ELY-1, produced on *E. coli* and phSE-5, produced on *S.* Typhimurium) to control *E. coli* and *S.* Typhimurium.

Phage ELY-1

Positive spot-test [6] in **9 strains** [2,3,4]

3 strains **infected**

Phage phSE-5

Positive spot-test [6] in **26 strains** [2,3,4]

3 strains **infected**

Methods

Bacterial Strains and Growth Conditions

E. coli [2] and ***S.* Typhimurium** (ATCC 13311) as phage hosts.

25 ºC in tryptic soy broth (TSB).

Bacteriophages

ELY-1 [5], **phSE-5** [6] and mixture of both **ELY-1/phSE-5**

Determining Bacterial Concentration and Phage Titre in Killing Curves

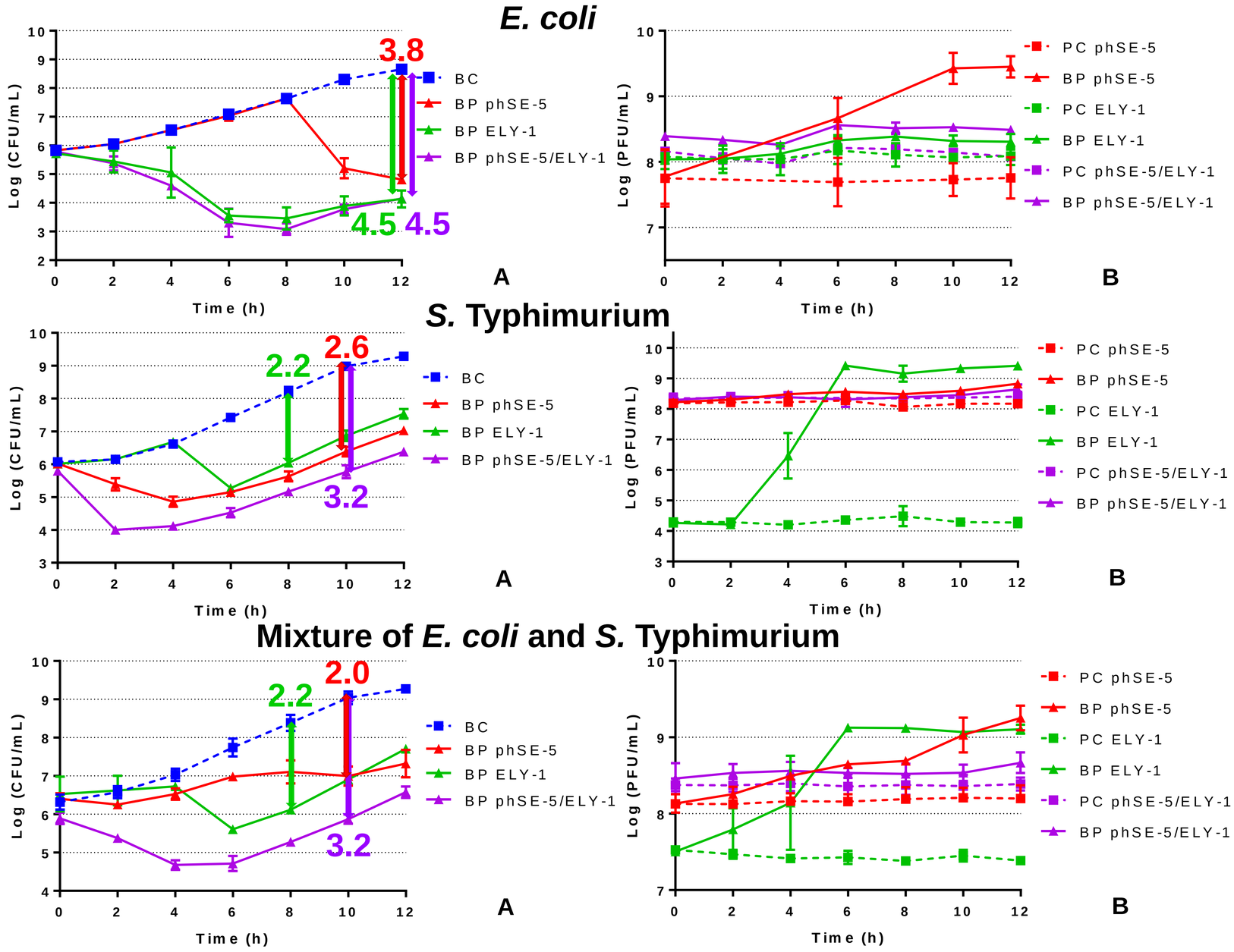
Pour plate and double-agar layer methods [7], respectively.

Results

Bacterial concentration (A) with (**BP**) and without (**BC**) phage

Phage concentration (B) with (**BP**) and without (**PC**) bacteria

E. coli



***S.* Typhimurium**

Mixture of *E. coli* and *S.* Typhimurium

- Bacterial mutants emerged at different rates [8] for different phages
- Bacterial *fitness* was **NOT** affected**, BUT** since it depends on several factors, **more** studies are **needed**

Conclusions

- A phage cocktail against Enterobacteriaceae has potential to be used prior to the identification of the pathogenic bacterium;
- Development of phage-resistant mutants was not prevented;
- Further studies are needed to understand the true potential of the use of these two phages and of the phage cocktail to control *E. coli* and *S.* Typhimurium, namely *in vivo* studies using animal models.

References: **[1] Costa, P. et al. Efficiency of Single Phage Suspensions and Phage Cocktail in the Inactivation of Escherichia coli and Salmonella Typhimurium: An In Vitro Preliminary Study. Microorganisms. 2019, 7(4), 94. [2] Alves, E. et al. Photodynamic inactivation of recombinant bioluminescent Escherichia coli by cationic porphyrins under artificial and solar irradiation. J. Ind. Microbiol. Biotechnol. 2008, 35, 1447–1454. [3] Silva, I. et al. Fate of cefotaxime-resistant Enterobacteriaceae and ESBL-producers over a full-scale wastewater treatment process with UV disinfection. Sci. Total Environ. 2018, 639, 1028–1037. [4] Pereira, S. et al. Potential of phage cocktails in the inactivation of Enterobacter cloacae—An in vitro study in a buffer solution and in urine samples. Virus Res. 2016, 211, 199–208. [5] Silva, Y.J. et al. Influence of environmental variables in the efficiency of phage therapy in aquaculture. Microb. Biotechnol. 2014, 7, 401–413. [6] Pereira, C. et al. Bacteriophages with potential to inactivate Salmonella Typhimurium: Use of single phage suspensions and phage cocktails. Virus Res. 2016, 220. [7] Adams, M.H. Bacteriophages; Interscience Publishers, Inc.: New York, NY, USA, 1959. [8] Filippov, A. et al. Bacteriophage-resistant mutants in Yersinia pestis: Identification of phage receptors and attenuation for mice. PLoS One 2011, 6, 1–11.**

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